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Investigation of Antioxidant Capacity of Several Luting Cements Processes by HPMC Method

SUMMARY

Background: *Free radicals (FR) occur in oral cavity where lot of food was transferred to through entire life under specific saliva conditions. Many enzymes, microorganism, alcohol beverages, nicotine and other harmful or indifferent substances when in contact to oral tissues might provoke oxidation process under specific condition creating FR's. The similar role might have various dental materials.* ***Aim*** *of the study was to record the level of antioxidant (AO) activity of several permanent (P) luting cements alone or combined with quercetin AO substance.* ***Materials/Methods:*** *P cements were Zn-phosphate, Zn-polycarboxilate, GIC and composite resin cement. They were prepared as original prescription and their variant by 1%weight addition of quercetin. AO activity of cements was measured by HPMC test evaluated by Student t test.* ***Results:*** *There were* statistically significant differences among *Zn-phosphate, Zn-polycarboxilate and resin dental cements ($p > 0,05$). GIC displayed significantly higher AO values ($p < 0,01$) versus other three cements. There were no difference in AO capacity between sample of original P cements and their corresponding quercetin variants ($p > 0,05$).* ***Conclusions:*** *Conventional GIC displayed the most powerful AO activity among P luting cements. Addition of 1% antioxidant quercetin did not improve AO capacity of investigated cements.*

Key words: Antioxidant, Antioxidant capacity, Free radicals, Luting cement, Glass ionomer cement, Eugenol, Quercetin, Flavone

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Introduction

An antioxidant (AO) is a molecule capable to inhibit oxidation of other molecules. During oxidation as chemical reaction, electrons are being carried from a substance to an oxidizing mean when free radicals (FR) could be produced. They could initiate chain reaction causing cell damage subsequently. Beneficial agents, AO molecules, could finish such chain reaction cleaning FR's intermediate products thus slowing down and repressing other oxidation reactions. Oral cavity is area where lot of food was transferred to throughout the life under specific wet conditions by the present of many enzymes, microorganisms, alcohol beverage, nicotine and other harmful or indifferent substances. All of them might be exposed to oxidation process under specific condition creating FR's. On the other hand, existed dental material within oral cavity might create FR's such as acrylics, dental alloys or side-effect products during bleaching or restorative/endodontic treatments. Consequently, humans could create enzyme or non-enzyme AO arrangement to prevent and protect tissue against FR's.

Considering the clinical steps before the temporary/permanent cementation procedure of the fixed restorations (creating the finishing demarcation, thread insertion and impression taking)[1] it is very important to take care of gingival health as well as architecture of the gingival margin. Subsequently, act of luting might be another harmful factor to slow down the surrounding tooth tissue healing[1]. Moreover, the presence of new implanted materials in oral environment might provoke creation of FRs as potential antagonist versus human defensive systems. This is the reason that several articles were published in past decade about chemical and physical

properties of dental biomaterials[2,3], as well as their toxic potential[4,5].

The damage caused by reactive oxygen species results in harmful consequences at the systemic level and to the pulpal/periodontal tissues. One of such common cases is peri-implantitis[5] triggered by gram-negative, anaerobic, and microaerophilic microbes producing reactive oxygen species that consequently stimulate proinflammatory cytokines.

Having in mind such trigger system it would be interesting to analyze the studies concerning potential substances and defense mechanism against FRs occurred in human body. One of them is Trolox^R, analogue of vitamin E, that exposes cytotoxicity lessening on the several studied biomaterials[6,7]. The study about insertion of less than 0,1% vitamin E to the base material maintains its initial physical and mechanical properties even exposes oxidation inhibition up to 24 months upon implantation[8].

The polymer chains that have natural AO's such as quercetin – flavone and curcumin, incorporated into a polymer backbone displayed attenuation on the material-induced oxidative stress[9]. Looking for the model that could be maintain AO level some authors used silica microspheres through a novel method by polyol-in-oil-in-water (P/O/W) emulsion and sol–gel methods[10]. Those authors added quercetin (in weight percentages of 5, 10, 15%) to an inorganic silica matrix in the synthetic process to create protective, bioactive, and biocompatible glass material in which the natural molecule firmly integrates into the inorganic network. Their results points out that higher quercetin amount were directly correlated to the higher AO effect.

Resuming various in vivo or in vitro methodologies applied in different researches it is to note that around 74 published articles could be extracted out of 407 combinations of samples and methods. Twenty-nine out of them were repeated in the consequent studies of which 19 were in vitro and 10 for in vivo models[11]. DPPH scavenging activity model was noted as the most applied as in vitro AO activity while lipid peroxide oxidation as in vivo. This article found the ethanol solvent agent for extraction purpose as frequent as possible[11]. However, it is to conclude that there is no accepted standard in the field of dental materials deals with AO activity. Namely, among numerous methods, there are very many choices considering in vivo/in vitro options, quality of sample and nature of liquid solvents. Our previous report about level of AO capacity on the several cements for temporary luting revealed more beneficial effect of eugenol-based materials in comparison to non-eugenol ones using ABTS method[12, 13]. The results and dilemma arisen of that study, as well as before mentioned citations, provoke us to aim the investigation about the level of AO activity of several luting cements alone or combined with antioxidant, recorded by direct current polarographic antioxidant assay.

Material/Methods

Material

The study material comprised four different types of luting cements for permanent bonding of the fixed restorations. The used commercially cements were original prescriptions as the next:

I. Cegal B- Zn-phosphate (ZP) cement (Galenika, Serbia)
II. Harvard Zn-polycarboxilate cement (ZPC) (Harvard Dent. Int., Germany)
III. Maxcem Elite- self-etch, self-adhesive resin cement (RC) (Kerr, Germany)
IV. Ketac Cem Easymix- Glass Ionomer luting cement (GIC) (3M ESPE, Germany)

The used antioxidant was encapsulated ≥98% (HPLC) powder of quercetin (Q) dehydrate (Sigma Aldrich, Co. LLC). Experimental cement mixtures are presented as before mentioned four (I-IV) preparations combined with quercetin powder forming the next groups: I_Q, II_Q, III_Q and IV_Q. Cement groups involved three samples each.

Methods

HPMC method (HydroxoPerhydroxo Mercury (II) Complex) is relative new method arranged by Physics Chemistry Institute (Belgrade, Serbia) and promoted in 2011[14]. Direct current polarography AO assay based on the decrease of anodic limiting current of Hydroxo Perhydroxo Mercury (II) Complex [Hg(O2H)(OH)] (HPMC) upon addition of AOs was applied. Using the polarography analyzer PAR (Princeton Applied Research), model 174A, equipped with an X-Y recorder (Houston Omnigraphic 2000), the current-potential (i−E) curves were recorded. A dropping mercury electrode (DME) with a programmed dropping time of 1 s was used as working electrode (a saturated calomel electrode-SCE as a reference) and a Pt-foil as auxiliary electrode. Starting 5 mM H_2O_2 concentration was obtained by addition of 100 μL of 1,00 M H_2O_2 into 20 mL of Clark Lubb's (CL) buffer (pH 9,8) (25 mL of 0,4 M H_3BO_3, 25 mL of 0,4 M KCl, and 40,8 mL of 0,2 M NaOH). Samples were gradually added into buffered H_2O_2 solution. Before each i-E curve recording, a stream of pure nitrogen was passed through the cell solution, during 5 min before the first recording and during 30 s after the addition of each aliquot. The inert atmosphere was kept by passing nitrogen above the cell solution. The initial potentials were 0.10 V and the potential scan rate was 10 mV s^{-1}.

The DME current oscillations were filtered with the low-pass filter of the instrument positioned for 3 sec. All experiments were done in triplicate at room temperature. Decrease of the anodic current of H_2O_2, that is initial i_l value (i_{l0}), obtained by recording 5 mM H_2O_2 solution, upon addition of investigated samples has been recorded. The remaining anodic limiting current (i_{lr}) obtained upon gradual addition of tested samples has been compared with the height of initial limiting current (i_{l0}). The percentage of i_l decrease has been calculated upon each addition of tested samples. Plots of percentage of i_l decrease calculated upon each addition versus added volume of samples. The slope of the linear part of the dose–response curves has been used as criterion of AO activity.

Statistic method of Student T test served to calculate the differences between the studied groups by confidential level of 0,05.

Protocol

Eight specific groups of cement mixtures, each by three samples, presented 24 samples for the study. The zinc phosphate (ZP), polycarboxylate (ZPC) and glass–ionomer (GIC) cements were consisting of powder (A) and liquid (B). Resin based dental cement (RC) consisted of pastes A (base) and B (catalyst). Variants of P luting cements were made by manual mixing of the original powder where 1% by weight was quercetin powder and corresponding amount of original liquid forming groups: I_Q, II_Q, III_Q and IV_Q.

The control of composition of investigated samples was obtained by precise weigh of their components by means of electronic analytic scale (Mettler PE360, Switzerland) and pipette for liquids. All investigated cements were hand mixed by plastic spatula in accordance to the manufacturer's instructions. After appropriate mixing time, the cements were placed into molds to produce cylindrical test specimens (6.0 ± 0.1mm high and 8.0 ± 0.1mm diameter). The mold was mounted on the glass slide and filled with cement material. Sheet of polyester film and a microscope slide was positioned on the top of it creating a flat surface. Excess material was removed by pressing over the brim of the mold. Tapping the mold by spatula and running the carefully mixing, the inclusions of air were minimized. The microscope slide was removed upon setting for I-III and I_Q-III_Q cement samples. The IV and IV_Q specimens were irradiated, both bases of cylinder, with external curing light source-450mW/cm (Visilux 2, Dental Products, 3M Company, USA) in three overlapping sections by 20 sec. Upon irradiation/setting, the specimens of all eight groups were removed from the mold and immersed immediately in 3 mL saline at 37°C. The specimens were removed from the saline after 24 h of dwelling and 0,5 mL extracts were analyzed for the AO activity.

Results

The results were presented in Figures 1 & 2. The slope of the linear part of the dose-response curves has been used as a criterion of AO activity by HPMC method.

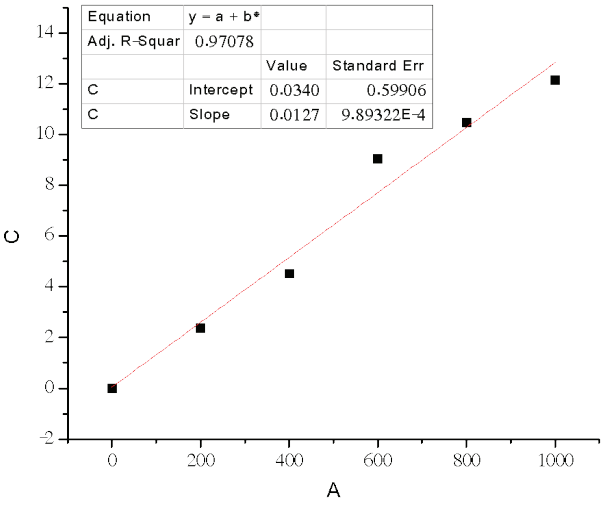

Figure 1. Slope value for ZP (Group I) Slope value- 0.0128±0.0010

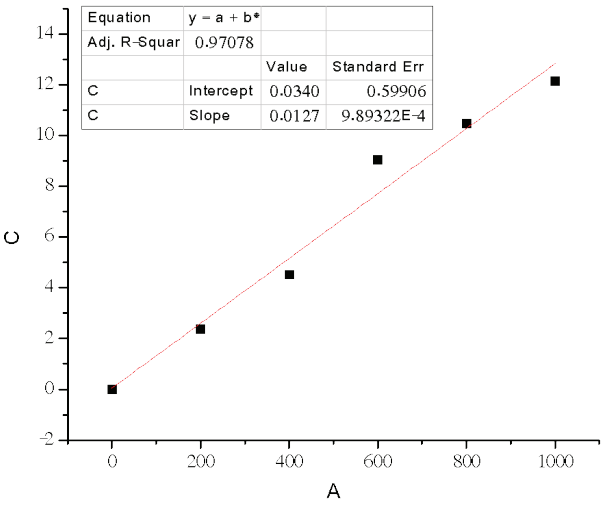

Figure 2. Slope value for ZP + Q (Group IQ) Slope value- 0.0130±0.0009

The analysis of AO capacity revealed statistically no significant difference among ZP, ZPC and RC ($p > 0,05$). On the other hand, GIC-KetacCem samples displayed significantly higher AO values versus other three original prescriptions **of P cements** ($p < 0,01$).

There were no difference in AO capacity between samples of original cement preparations and their corresponding quercetin variants [I vs I_Q, II vs II_Q, III vs III_Q and IV vs IV_Q) ($p > 0,05$)].

There were no statistical significant difference in AO activity among the next quercetin groups, samples (I_Q, vs II_Q and III_Q, II_Q vs III_Q) ($p > 0,05$, Table 1).

Table 1. Antioxidative activity of luting cement registered by HPMC method through the mean slope values. Percentage of decrease of HPMC anodic limiting current (i_l) vs volume of gradually added samples (μL)

Cement group	I (ZPC)	II (PCC)	III (RC)	IV (GIC)	I_Q	II_Q	III_Q	IV_Q
Slope value (mean)	0,0128	0,0133	0,0095	0,0170	0,0130	0,0137	0,0097	0,0174

Discussion

The materials for definite fixation of cast restorations were chosen as the frequent applicable in nowadays dental practice. All of them present specific material regarding composition (Zn-phosphate, Zn-polycarboxilate, glass ionomer and resin dental cements). They are chosen due to the fact that different chemical reaction of setting all of them might have influence to the creation of free radicals, responsible for oxidative stress. HPMC polarographic assay was confirmed as valuable test because revealed the similar relationship between eugenol and non-eugenol cement materials (our yet unpublished results) as ABTS test did in our previous study from 1998[6].

The solvent we used in this study was saline solution chosen due to its osmolality near the human sera that is similar to media of dental pulp and periodontal tissues where potent odontoblasts, cementoblasts, osteoblast and other pluripotent cells live. Unfortunately, saline as the most appropriate solution to the vital tissue, what was our intention of mimicking pulpal/periodontal environment, is one of the weakest solvent in comparison to alcohol which is the most used liquid in AO effect studies according to bibliometric study[1]. Although we did not use opportunity of both methods to follow the AO activity change in the course of time, considering that fact, one can say that both applied measuring analytical quantitative methods we used (ABTS and HPMC) were simple and comfort for the study of that kind. In addition, antioxidant substances (quercetin, vitamins C and E) exposed high level of AO activity[1,7,10] it might be explained by their high dose (quercetin 5, 10 and 15%) that could be detrimental to other physico-chemical of materials prognoses to exist for several years in wet media of saliva and withstand to occlusal stresses and oral metabolites.

The whole protocol of our study was imagined to imitate oral environment where dwelling time of 24 h presents the period of prolonged setting of used cements. However that is the period when dental pulp and tooth surrounding tissue suffer too much. Also, it is important fact that subsequent gingiva and vital pulpo-dentinal complex injury are caused by therapeutic manipulations and due to the consequences of chemical setting reactions are most expressed as the patient' s trouble in the first 24 h upon cementing (unfinished chemical reactions, surplus of eugenol content, elution of PMM out of composite resin dental cements etc.).

As the seventh day present the standard point in protocolar dental materials' features, it will be interesting to follow and record the AO activity at that time point (our running study) expecting the decrease of amount of FR's whether of saliva enzymes action or finishing of setting of used luting cement.

The low activity at RC samples might be explained by the fact that composite resin polymerizes by free radicals polymerization generated when a photo-initiator (camphorquinone) absorbs light energy (photons) emitted from the curing light initiates polymerization by reacting with a photoreducer when tertiary amine form FRs thus initiating crosslinking[15].

Our result about higher level of AO effect by HPMV method of Ketac Cem (Glass Ionomer Cement) regarding Zn-phosphate and polycarboxylate has no explanation. If speculate that AO capacity is inverse correlation to the cytotoxicity level, the results of Trumpaite-V[16] where Zn-phosphate exposed as lower cytotoxic then Fuji Plus RMGIC, are in discrepancy to the ours. Unfortunately, our results of AO capacity are not comparable to the results of other studies due to the difference in laboratory methodologies and sample preparation. Also it is to note that there is no accepted standard among investigators about choice of used device for measuring AO capacity, solvent agent, time of experimental release of AO species and their detection whether in vivo or in vivo system. For example, review bibliometric article about parameter that influence the results about AO effect point out to the organic solvents (alcohol, acetone) as the most effective[1]. Although they have high solvation potential their similarity concerning osmolality, is too far from biological saliva and tissue liquor. Therefore inorganic solvents might be more appropriate for in vitro while organic for in vivo experiments.

Moreover, the investigations of this kind necessitate wide spectrum of tasks considering the type of carrier of AO agent. One of the study reports about silica-gel carrier[17] as inert substance with depot character where quercetin exposed AO activity but at high concentration (5, 10 and 15%). Nevertheless, such material composition will convert itself into the variant of worsen physico-chemical features meaningful for long-lasting cast restorations. The similar is for AO's where their rise provokes lessening level of cortisol[13].

Although Soheili et al.[18] investigated in vitro effects of ascorbate and Trolox on the biocompatibility of dental restorative materials, the further progress of the studies

about AO agents should be focused rather as in vivo model. Our researches, that are under way, is targeted to find the balance between AO partition and other cement ingredients that will result in long-lasting antioxidant releasing in saliva medium approved through in vivo tests.

Conclusions

Conventional GIC Ketac Cem displayed the most powerful AO activity among studied original cement prescriptions. Addition of antioxidant materials (quercetin – flavone) in amount of 1% by weight did not improve AO capacity among all four investigated original cement preparations in media of saline solution.

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